*J. Environ. Nanotechnol.*
*Volume 4, No.2(2015) pp. 37-46*
*ISSN (Print): 2279-0748*
*ISSN (Online): 2319-5541*
*doi:10.13074/jent.2015.06.152149*

# Microbial Synthesized Silver Nanoparticles for Decolorization and Biodegradation of Azo Dye Compound

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*Received: 13.04.2015 Accepted: 27.05.2015*

## *Abstract*

*Biological approach for silver nanoparticle synthesis using microorganisms like Bacteria, Fungi, algae and plants have received profound interest because of their potential to synthesize nanoparticles of various size, shape and morphology. The present research work focus on bacterial synthesis of silver nanoparticles their efficiency forbioremediation of Congo red dye. Twenty one types of different bacterial species have been isolatedfrom the different industrial waste effluent and bacterial consortium was prepared. Potential silver resistant bacteria were isolated from bacterial consortium using scale up method and identified as Bacillus pumillus by Gram staining, biochemical tests and genome sequencing method. After that Silver nanoparticles were synthesized using potential silver resistant bacteria. Silver nitrate and silver sulfate were used as precursor at different concentration for silver nanoparticle synthesis. Color change from colorless to brownwas observed synthesis of silver nanoparticles was further confirmed by UV Visible spectroscopy. The silver nanoparticles formation was (5-93 nm in size) also confirmed by Transmission electron microscopy, Particle size analyzer, Dynamic light scattering depending on the concentration of silver precursor.In this research, we have shown for the first time the use of Bacillus pumillis for synthesis of silver nanoparticles. Dye decolorization and biodegradation was studiedusing silver nanoparticles, silver resistant bacteria and silver resistant bacteria influced by silver nanoparticles separately. Nano based bioremediation was found 13 % efficient than the microbial remediation. Thus the developed nano-bioremediation technology is biocompatible, simple and reliable method and can be applied to decolorize dye as well as antimicrobial agent.*

***Keyword:*** *Bioremediation; Environmental Pollution; Nano science; Silver nanoparticle; Nano-bioremediation.*

## 1. INTRODUCTION

Industrial revolution for fulfilling the demands of increasing population during production, results in pollution of water, air and soil. The discharge of pollutants from various industries poses threat to the surrounding environment. The industries like textile and paper large quantities ofwater and produces huge volume of waste water fromdifferent steps in the dyeing and finishing process. Different types of dyes are used in paper, leather, textile, cosmetics industry. Among all dyes,azo dyes are largest and most versatile class of dyes and are widely used in textile industries. More than 2000 structurally different azo dyes are currently in use. These dyes account for approximately 60-70 % of all dyes used in food and textile manufacture. Several physico-chemical methods such as adsorption, chemical treatment and ion pairextractions have been adopted and proven to be costly while producing large amounts of sludge and thus they also lead to second pollution. To overcome such problems research has been moving toward biological methods as these methods are eco-friendly and cost effective. Many microorganisms like bacteria, fungi have been reported to solve the problems regarding environmental remediation (Da-Guang, 2007). But as the load of pollutants in environment is very high, the bioremediation alone is not sufficient. So some technology has been developed to enhance the bioremediation process like Nano-bioremediation. Nano-bioremediation technology includes the

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application of nanoparticles or nanotechnology to enhance the process of bioremediation.

Biological synthesis process provides a wide range of environmentally acceptable methodology, rapid, cost effective, eco-friendly and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications which include catalysts in chemical reactions (Krolikowska *et al.* 2003), bio labeling, antimicrobial agent, electrical batteries (Klaus-Joerger, *et al.* 2001) and optical receptors (Parashar, *et al.* 2009;VibhaSaklani *et al.* 2012). Microbial source to produce the silver nanoparticles shows the great interest towards the precipitation of nanoparticles due to its metabolic activity. Ofcourse the precipitation of nanoparticles in external environment of a cell, it shows the extracellular activity of organism. But the particles should be chemically stable without under degradation such as partial oxidation (Berger, 2006).The first evidence of bacteria synthesizing silver nanoparticles was established using the *Pseudomonas stutzeriAG259* strain that was isolated from silver mine. There are some microorganisms that can survive metal ion concentrations and can also grow under those conditions, and this phenomenon is due to their resistance to that metal. The mechanisms involved in the resistance are efflux systems, alteration of solubility and toxicity via reduction or oxidation, bio-sorption, bioaccumulation, extracellular complex formation or precipitation of metals, and lack of specific metal transport systems (Haefeli *et al.* 1984). To analyze the effect of biologically synthesized silver nanoparticles over bioremediation research work have been done. As silver act as antimicrobial agent, silver resistant bacteria was isolated from industrial waste effluent and identified. Silver resistant bacteria were used to synthesize silver nanoparticles and decolorization and biodegradation of Congo red azo dye compound. Similarly, dye decolorization and biodegradation was also studied using microbial synthesized silver nanoparticles and potential silver resistant bacteria influenced by silver nanoparticles. During the research work the effectiveness of nano based remediation was studied.

## 2. MATERIALS & METHODS

### 2.1 Collection of Samples

Twelve samples were collected from effluent disposal sites of different industries located in Naroda GIDC phase I to IV, Ahmadabad, Gujarat, India in the month of December 2013. Industrial waste effluents from different industries like Dye, Textile, Chemical, Electronic, paper, Ceramic etc. were taken. Samples were collected in sterile plastic bottles having capacity of two liters. Temperature and pH were measured at the time of sampling and BOD samples were collected in the BOD bottle at the time of sampling.

## 3. PHYSICO-CHEMICAL AND BIOLOGICAL ANALYSIS OF WASTE WATER

Physico chemical and biological analysis of samples was done using the standard methods

### 3.1 Chemicals and media

The nutrient broth (N Broth) at pH 7 used during study contained: 0.5% Peptone 0.3% yeast extract 0.5% NaCl and distilled water. The culture media was autoclaved at 121 ºC for 15 min. The Minimal Salt Broth used during study contained: Glucose–0.57 g, Sucrose–0.285 g, Mono Potassium Phosphate–0.2 g, Di Potassium Phosphate– 0.7 g, Sodium Citrate– 0.05 g, Magnesium Sulfate – 0.010 g, Water – 100 ml, pH – 7.

## 4. BACTERIAL ISOLATION AND IDENTIFICATION

The bacteria were isolated from different Industrial waste effluents collected from Naroda G.I.D.C., Ahmadabad. Serial dilutions (up to $10^{-10}$) of samples were inoculated into nutrient agar medium by spread plate technique. Pure colonies of bacteria were isolated and identified using colony characteristics, gram staining and biochemical test methods. Then all the different bacterial colonies were mixed in sterile distilled water and consortium was prepared having 21 different types of bacteria.

## 5. ISOLATION OF POTENTIAL SILVER RESISTANT BACTERIA USING SCALE UP TECHNIQUE

1 ml sub cultured bacterial consortium was inoculated into Erlenmeyer flasks (250 ml) containing minimal salt concentration and no silver. The inoculated flasks were kept in an orbital shaker incubator at 160 rpm, 30 ºC. for 7 days. After 7 days, 1 milliliter of this culture media was taken and added to a minimal salt broth having silver concentration of 10 mg/l, the flasks were again kept on orbital shaker incubator at 160 rpm, 30 ºC for another 7 days.

Likewise, the microbial culture was sub cultured into minimal salt broth media with a silver concentration of 25 mg/l and 50 mg/l and 75 mg/l and 100 mg/l was kept on orbital shaker incubator at 160 rpm, 30 ºC for increasing a total period of 42 days

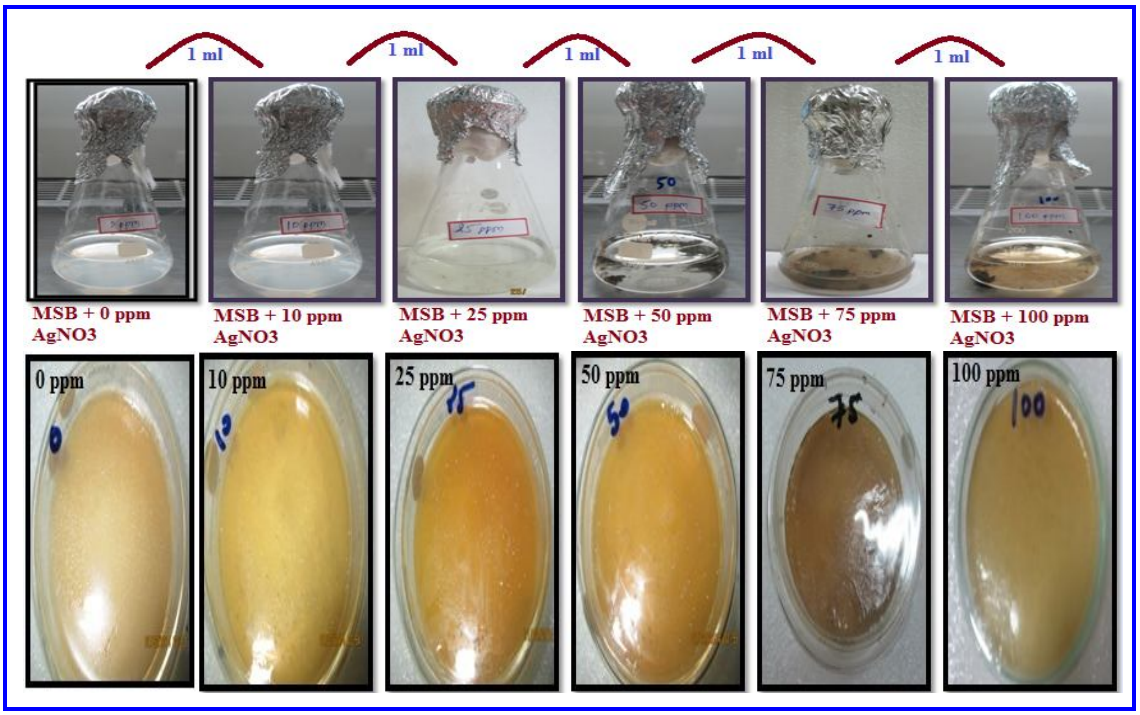


**Fig. 1: Scale up technique**

at the frequency of 7 days for interaction of microorganisms with the compound. At every interval the 0.1 ml of minimal salt broth was taken and inoculated on minimal agar plates having the same concentration of silver to isolate colony. At this stage, the bacteria were found adapted to silver nitrate and by assessing the silver nitrate (Razia Khan *et al.* 2013).

## 6. IDENTIFICATION OF POTENTIAL SILVER RESISTANT BACTERIA

Silver resistance bacteria have been isolated than pure culture was prepared and further identified using-Biochemical test, Gram staining and Full Genome sequencing method.

## 7. SYNTHESIS OF SILVER NANOPARTICLE USING ISOLATED SILVER RESISTANT BACTERIA

Silver resistant bacteria were isolated from the sample. Suspension of pure culture of bacteria was prepared. A loop full of suspension was inoculated in Erlenmeyer flasks containing 100 ml sterile Nutrient broth in each. Different concentration of silver nitrate like 1 to 5 mM was added. The flasks were incubated in dark as well as some in bright condition for 24 hours at 37 ºC. (Vidhya Lakshmi Das *et al*., 2014).

## 8. SYNTHESIS OF SILVER NANOPARTICLES USING BACTERIAL SUPERNATANT

Bacteria were grown in a 250 mL Erlenmeyer flask that contained 100 mL of nutrient medium. The flasks were incubated for 24 h in a bacteriological incubator shaker set at 120 rpm and 30° C. After the incubation period, the culture was centrifuged at 8000 rpm and the supernatant used for the synthesis of AgNPs. 1 mM of $AgNO_3$ was mixed with 5 mL of supernatant in a 250 mL Erlenmeyer flask. Bio-reduction was monitored by recording the UV–visabsorption spectra as a function of time of the reaction mixture (Jeevan *et al.*2012).

## 9. PURIFICATION OF SILVER NANOPARTICLES

The particles were washed two times with distilled water and three times with ethanol to remove bacterial cell debris by centrifugation at 8000 rpm for 10 minutes. Then silver nanoparticles were dried in hot air oven for 15 to 20 hours at 50 ºC (Faghri Zonooz and Salouti, 2011).

## 10. CHARACTERIZATION OF SILVER NANOPARTICLES

The biosynthesis of silver nanoparticles by *Bacillus pumillus* was supervised visually. The absorption spectra of the reaction mixture of $AgNO_3$ and aqueous extract were analyzed by the UV–Visible spectrophotometer (Halo DB) in the range of 250–750 nm. Further characterization was done using Particle size analyzer (Malvern S90), Dynamic light scattering, Transmission electron microscope (TEM-TECNAI 200 Kv TEM (Fei, Electron Optics)) (Rati Ranjan Nayak *et al*. 2011) and Fourier transform infra-red spectroscopy (Model no. FTIR (Perkin Elmer) Spectra).

## 11. DECOLORIZATION AND BIODEGRADATION OF CONGO RED DYE

The reactive dye used in this study was Congo red dye. An analytical grade Congo red obtained from Sigma Aldrich was utilized in this study (Palanivelan, *et al.* 2012).

### 11.1 Medium

Chemicals and medium ingredients used for this study were of analytical grade; purchased from Hi-media laboratories nutrient agar broth containing (gram/liter) Peptone- 5 g, Sodium chloride- 5g Beef extract- 1.5 g, Yeast extract 1.5g Final pH 7.4.

For comparative study, three experimental set ups were prepared. In first set up only Silver resistant bacteria were inoculated, in second set up silver nanoparticles and Silver resistant bacteria were inoculated while in third experimental set up only 50 ppm Microbial synthesized silver nanoparticles were added having average size of 40 nm.

## 12. EFFECT OF DIFFERENT CONCENTRATIONS OF DYE ON DECOLORIZATION

To check the efficiency of dye decolorization by the bacterial isolate and silver nanoparticles, decolorization assay was carried out at different concentration of Congo red dye, 10 mg/L, 25mg/L, 50 mg/L, 75 mg/L, and 100 mg/l. It is reported that Congo red dye shows its maximum absorbance at 498 nm (Pooja Mahajan and Jyotsna Kaushal, 2013). A 5.0 ml aliquot of the decolorized culture broth was placed in Eppendroff tubes and centrifuged at 8000 rpm for 10 minutes. The supernatant was recovered and analyzedspectrophotometrically at a wavelength corresponding to the maximum absorbance of the dye which is 498 nm. The uninoculated medium was used as control and the medium without dye was used as blank. The efficiency of dye degradation / decolorization can be measured using the absorbance value taken at different time intervals. To find the decolorization activity difference between final absorbance and initial absorbance was measured of same samples having dye after incubation. The efficiency of the isolates to degrade/decolorize Congo red (Decena and Barraquio,2004) was expressed as:

**Calculation**

$$\text{Congo red dye decolorization} = \frac{\text{Initial Conc. of dye} - \text{residual conc. of dye}}{\text{Initial Conc. of dye}} \times 100$$

## 13. RESULT & DISCUSSION

Physico chemical analysis data show the exceed value of Alkalinity, acidity, chloride, hardness, TOC and COD. Microbial characterization done by serial dilution method showed presence of diverse classes of microbes such as *E.coli., Bacillus sp., Enterobactor sp., Serratia sp., Micrococcus sp., Klebsiella sp., Shigella sp. and proteus sp.*. etc.. Twenty one different types of bacteria have been isolated from the industrial waste effluent. Sample no-2 collected from Mayur dye stuff industry shows the absence of microorganisms because of high amount of toxic chemicals. Sample No: 11 collected from Municipal bore well, shows the high diversity of the microorganisms.

## 14. IDENTIFICATION OF POTENTIAL SILVER RESISTANT BACTERIA

In the serial dilution method, bacterial consortium having twenty one types of different bacterial species were inoculated to increasing concentration of silver nitrate from 10 to 120 ppm in shaking condition at 30 °C. The potential silver resistant bacteria resist upto 100 ppm concentration of silver nitrate was isolated. According to colony characteristic, gram staining, biochemical test and whole genome sequencing method the potential silver resistant bacteria was identified as *Bacillus pumills.*

## 15. SILVER NANOPARTICLE SYNTHESIS AND CHARACTERIZATION

Silver nanoparticles (AgNPs) were synthesized using different method and different concentration of precursor. This shows different size and characteristic. The Erlenmeyer flasks with silver resistant bacteria or bacterial supernatants were a white color before the addition of Ag+ ions and this

changed to a brownish color on completion of the reaction with $Ag^{+}$. The appearance of a light brown color in solution suggested the formation of silver nanoparticles.

## 16. UV VISIBLE ANALYSIS OF SILVER NANOPARTICLE

Reduction of silver ions into silver nanoparticle during exposure to bacteria and bacterial suspension were observed as a result of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticle were observed around 430 and 420 nm.

## 17. EFFECT OF SILVER ION CONCENTRATION

The UV-vis spectrum shows effect of silver nitrate concentration in the silver nanoparticles synthesis by using the supernatant from silver resistant bacteria. Characteristic Surface Plasmon absorption band was observed at 440 nm for the brown colored silver nanoparticles synthesized from 1 mM silver nitrate. At the 1 mM concentration shows narrow band with increased absorbance whereas other concentrations shows broad peak at 440 nm. The absorption was decreased while increasing the concentration of silver ions from 1mM to 5 mM.

**Fig. 2** illustrates the absorbance values of silver nanoparticle solution using UV- visible spectrophotometer. As the concentration of precursor silver nitrate increased the peak value of absorbance also found to be increased.

## 18. PARTICLE SIZE ANALYZER

Microbial synthesized silver nanoparticles were suspended in sterile distilled water and sonicated for 10 minutes for particle size analysis using Particle size analyzer. Particle size analyzer gives analysis data for the average size as well as distribution of silver nanoparticle. Qualitative and quantitative analysis of silver nanoparticle sample has been done by using this instrument.Different concentration of precursor was taken to analyze the effect of concentration over size of silver nanoparticles.

## 19. DYNAMIC LIGHT SCATTERING (DLS) ANALYSIS

Same AgNPs samples were also analyzed using Dynamic light scattering analyzer to find the zeta potential value, polarity, conductivity, charge and mobility of synthesized silver nanoparticles. Dynamic light scattering (DLS) is a technique for characterizing the size of colloidal dispersions which utilizes the illumination of a suspension of particles or molecules undergoing Brownian motion by a laser beam. The time-dependent fluctuations in the intensity of scattered light that occur are analyzed using an auto correlate which determines the autocorrelation function of the signal (Umoren *et al.* 2014).

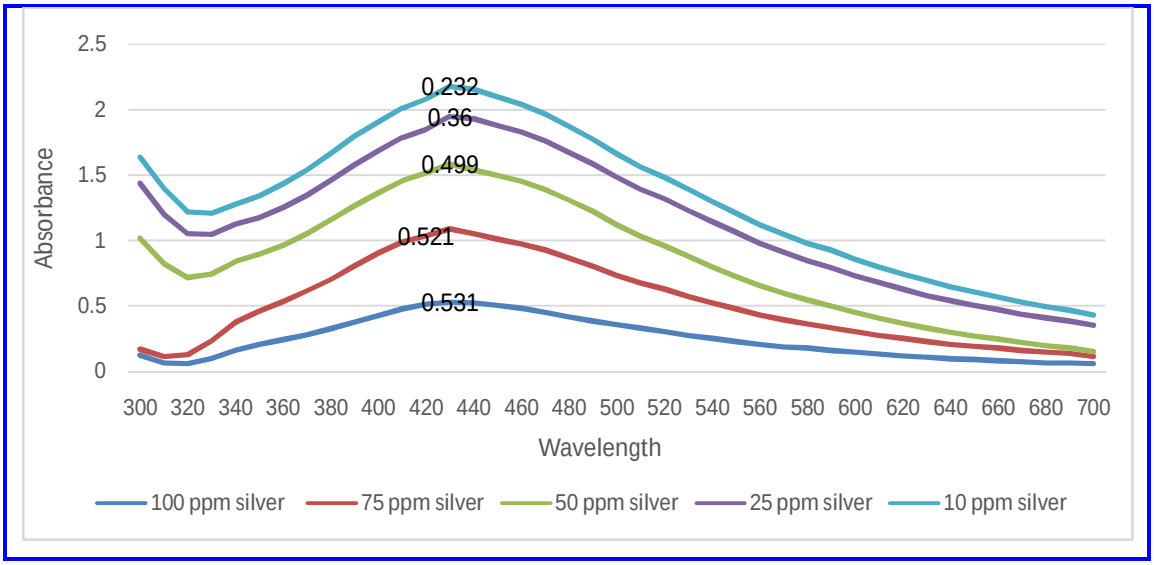


**Fig. 2: Absorbance Spectra of AgNPs**

**Table 1. PSA and DLS Analysis for AgNPs synthesized using different concentration of $AgNO_3$**

| PSA &DLS Analysis | 1 mM | 2 mM | 3 mM | 4 mM | 5 mM |
|---|---|---|---|---|---|
| Size ~ | **5.835** | **28.46** | **49.85** | **78.12** | **93** |
| PDI | 0.352 | 0.280 | 0.249 | 0.287 | 0.352 |
| Intercept | 0.879 | 0.897 | 0.920 | 0.892 | 0.892 |
| Mobility u/s/V/cm | -1.26 | -0.98 | -1.88 | 1.91 | -1.64 |
| Zeta Potential mv | 16.10 | -12.56 | -24.04 | 24.45 | -20.9 |
| Charge C | 0.023 | 0.036 | 0.0330 | 0.045 | 0.031 |
| Polarity | Negative | Positive | Negative | Positive | Positive |
| Conductivity µS/cm | - 50 | 93 | 159 | 59 | 112 |

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The Ag-NPs obtained possess positive or negative zeta potential value. Zeta potential is an essential parameter for characterization of stability in aqueous Ag-NPs suspensions. A minimum of ±30 mV zeta potential values is required for indication of stable nanosuspension (Kamyar Shameli *et al.* 2012). Due to lack of stabilizing agent, above all nanoparticles show incipient stability. Negative polarity indicates basicity of medium while positive polarity shows acidic nature of solution having nanoparticles. PDI(Poly Dispersity Index) represent the monodispersity of silver nanoparticles. It should be below 1. All the silver nanoparticles showed good monodispersity.

## 20. TRANSMISSION ELECTRON MICROSCOPE (TEM) ANALYSIS

To know the actual size and shape of the silver nanoparticles, samples were systematically analyzed by TEM. Conventional TEM micrographs were recorded on a Transmission electron microscope (TEM-TECNAI 200 Kev TEM (Fei, Electron Optics). The obtained particulates were dispersed in suitable solvent and then deposited on the carbon coated grid for TEM studies.

TEM images confirm the presence of roughly spherical and roughly rod shaped silver nanoparticles synthesized using 2 mM silver nitrate. The TEM images and their size distributions revealed that, the mean diameters of Ag-NPs were about size range from 20.57 to 53.00 nm and average size 43 nanometer in diameter having roughly oval in shape. Due to higher concentration, agglomerations of silver nanoparticles were also found.

### 0.002 M Silver Sulfate

Silver sulfate was taken as silver precursor for AgNPs synthesis. Silver nanoparticles have been synthesized using different concentration of silver sulfate. As the silver sulfate contain two ions of silver, it was found that bacterial enzyme could not reduce properly as silver nitrate.

TEM images confirm the presence of roughly spherical and roughly rod shaped silver nanoparticles synthesized using 2 mM silver sulfate solution. Figure 4-A shows the image for agglomerates of small grains silver nanoparticles. The particle size histograms of silver particles show that the particles range in size from 27.60 to 59.42 with mean diameter 43.51 nm. Figure 4-B illustrates the silver nanoparticle with width approximately 29.08 nm.

## 21. FOURIER TRANSFORM INFRA RED SPECTROSCOPY (FTIR) ANALYSIS

FTIR spectroscopy was used to identify the functional groups present in samples. Silver nanoparticles were suspended in sterile distilled water and sonicated for 20 minutes prior to analysis.

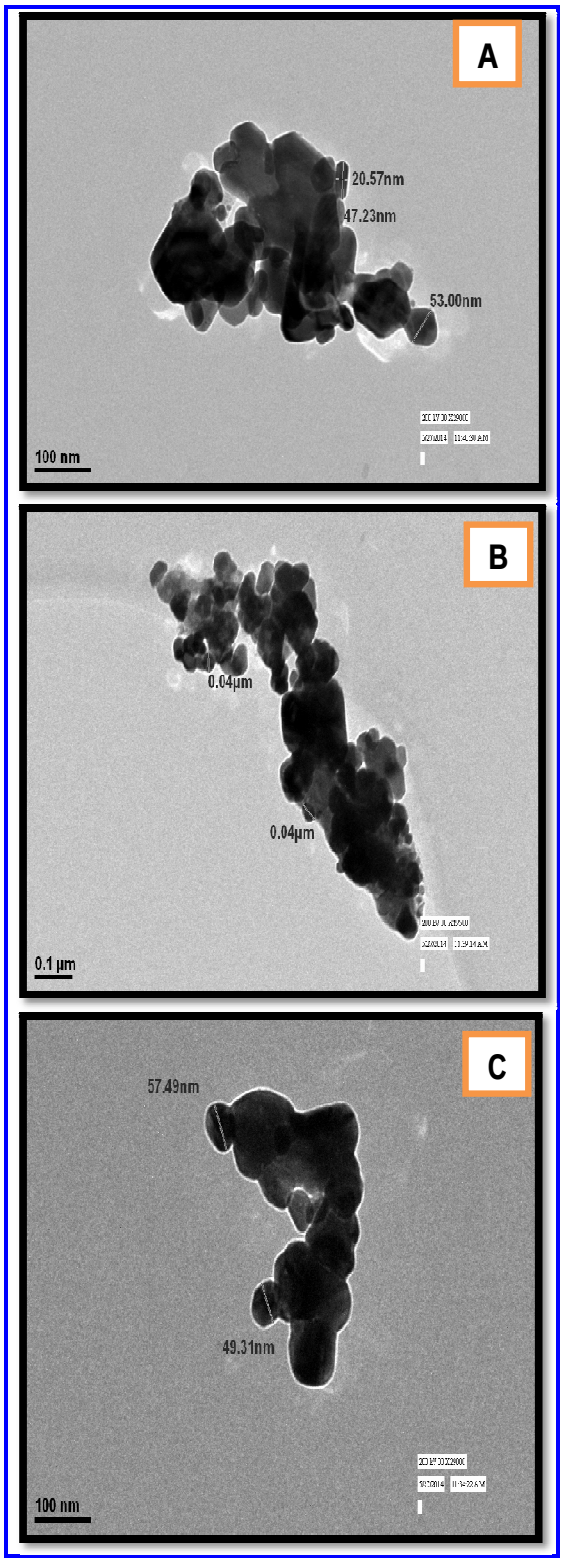


Fig. 3:A to C TEM image of silver nanoparticles and its particle size distributions.

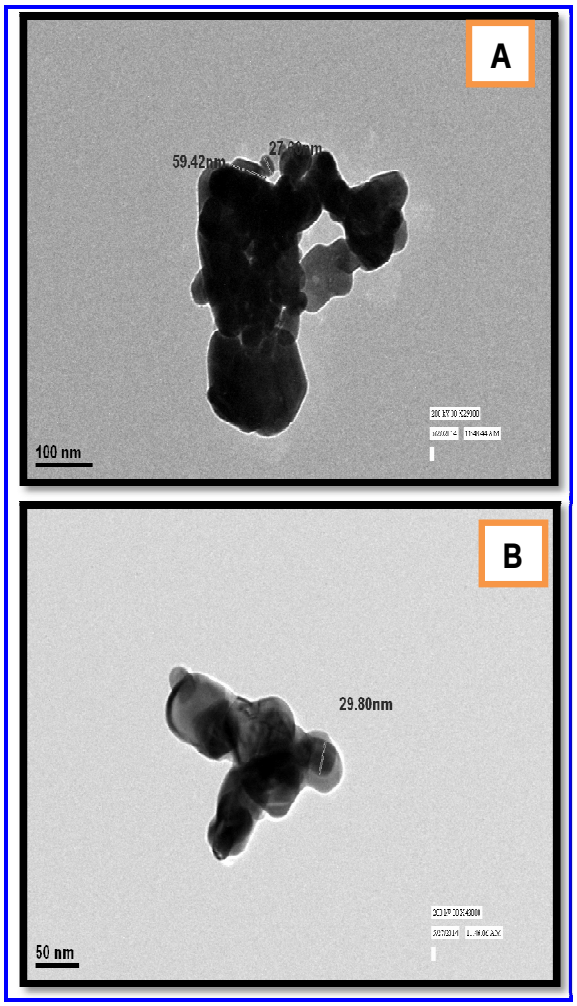


Fig. 4: A, B- TEM image of silver nanoparticles and its particle size distributions.

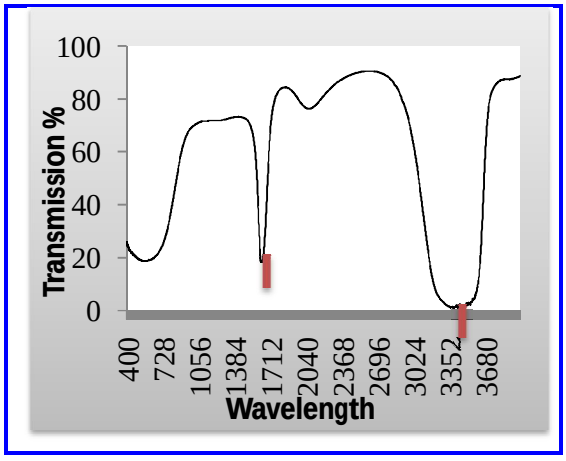


Fig. 3: Fournier Transform Infra red spectroscopy transmission analysis

FTIR figure displayed the presence of hydroxyl functional group which was found by peak transmission value at 3200-3500 $cm^{-1}$. Due to silver nanoparticles were suspended in distilled water, hydroxyl group was found. Alkene group was found between1620-1680$cm^{-1}$. Whereas the stretch for C-Br (Organo bromine) were found at 528 $cm^{-1}$. Organo bromine and Alkeme group was found due to the presence of bacterial enzymes or metabolites.

## 22. CONGO RED DECOLORIZATION AND DEGRADATION

Congo red dye decolorization and degradation analysis have been done using isolated silver resistant bacteria, combined activity of Silver resistant bacteria and silver nanoparticles which is also known as nano-bioremediation as well as only silver nanoparticles. To study the nano bioremediation, silver nitrate as silver nanoparticle precursor was added within the growth medium after autoclaving the growth medium. SRB synthesized silver nanoparticles within 2 hours and showed their activity to decolorize dye. In this experimental analysis the different concentration i.e. 10 to 100 ppm of Congo red dye was taken to check the decolorization and biodegradation activity. While concentration of silver 20 ppm was kept constant. Congo red dye shows the highest absorbance at 498 nm. Due to this absorbance was taken at 498 nm up to 72 hours at time interval of 12 hours.

Silver resistant bacteria could decolorize Congo red dye having 10 ppm concentration up to 83.96 %, AgNPs alone could decolorize only 45.12 %, of dye having 10 ppm concentration, while silver resistant bacteria within AgNPs can decolorize the same concentration of dye upto 96.99 % within 72 hours of incubation at 30 ºC at static condition. While SRB decolorized the Congo red dye having 100 ppm concentration up to 51.49 % AgNPs alone could decolorize only 26.35 % while silver resistant bacteria within AgNPs can decolorize the same concentration of dye up to 60.92 % within 72 hours of incubation at 30 ºC at static condition. Among all analysis silver resistant bacteria under the influence of silver nanoparticles showed the highest dye decolorization and degradation ability than others. Due to bacteria were isolated from psychrophilic environment, 30 ºC temperature was maintained during the experiment. The resulting data represents that bacteria and silver nanoparticles has the highest capacity for dye degradation and decolorization. It was found that incubation condition like temperature, pH etc..played crucial role in dye removal process.

## 23. EFFECT OF CONCENTRATION OF DYE

All the data indicate that as the concentration of dye increased from 10 ppm to 100 ppm, the decolorization and degradation ability of bacteria was also found decreased. Due to higher concentration, the bacterial enzymes could not reduce the dye properly.In the case of silver nanoparticles alone showed lowest dye removal ability as increased concentration due to limited adsorption area.

## 24. CONGO RED DYE DEGRADATION

### 24.1 UV Visible Spectroscopic Analysis

Dye degradation analysis has been done to check the quantity of dye present in samples within the passage of time using the same samples which were taken for Congo red decolorization study. Congo red dye shows its higher absorbance between 495 to 520 nm. The absorbance was taken between 250 to 750 nm for samples incubated for different time of intervals, having different dye concentration. Within the passage of the incubation time, the peak value was found decreased between 490 to 520 nm. Decreased absorbance value indicated the dye degradation.

### 24.2 Total Organic Carbon Analysis

As the Congo red dye is an organic compound, within the passage of incubation time organic carbon was found to be decreased and inorganic carbon was increased indicated the dye degradation. For 50 ppm Congo red dye concentration, when incubated with bacteria and silver nanoparticles, the initial concentration of organic carbon was found 1.06 g/l while inorganic carbon was found 14.54 mg/l. after 72 hours TOC was decreased to 452.7 mg/l while inorganic carbon was increased to 308.2 mg/l.

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**Table 2. Percentage Value for Comparative Dye Decolorization**

| | 10 ppm Dye | | | 25 ppm Dye | | | 50 ppm Dye | | | 75 ppm Dye | | | 100 ppm Dye | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | B+ Ag | AgNPs | B | B+ Ag | AgNPs | B | B+ Ag | AgNPs | B | B+ Ag | AgNPs | B | B+ Ag | AgNPs |
| 8 H | 10.3 | 11.69 | 8.44 | 9.07 | 11.5 | 7.41 | 6.53 | 7.52 | 5.99 | 4.77 | 6.2 | 4.27 | 4.27 | 4.92 | 3.31 |
| 12H | 18.2 | 31.1 | 17.7 | 16.6 | 30.4 | 15 | 14 | 22.3 | 11.1 | 12.6 | 15.4 | 7.26 | 9.49 | 13.8 | 5.14 |
| 24 H | 36.79 | 44.85 | 25.81 | 27.59 | 40.95 | 22.1 | 24.07 | 32.33 | 18.38 | 19.83 | 30.29 | 13.85 | 17.9 | 27.14 | 10.95 |
| 36 H | 47.16 | 58.3 | 28.89 | 43.66 | 47.98 | 26.13 | 43.51 | 43.07 | 22.51 | 34.65 | 40.8 | 18.4 | 28.36 | 35.47 | 13.39 |
| 48 H | 63.5 | 74.92 | 34.7 | 59.54 | 68.2 | 31.82 | 57.5 | 63.7 | 30.6 | 48.2 | 54.5 | 28.8 | 40.4 | 45.02 | 25.7 |
| 60 H | 71.38 | 83.09 | 41.88 | 66.72 | 79.52 | 34.86 | 60.53 | 68.62 | 32.43 | 51.56 | 62.76 | 30.43 | 46 | 54.82 | 26.35 |
| 72 H | 83.9 | 96.99 | 45.1 | 76.93 | 84.1 | 36.41 | 67.8 | 73.6 | 33.8 | 58.6 | 69.7 | 30.0 | 51.4 | 60.92 | 26.3 |

## 25. CONCLUSION

According to physico-chemical analysis data industrial waste water exceed the standard limit. Very high quantity of pollutants was present. Among the number of synthesis methods for silver nanoparticle, biological method provides environmental friendly, simple, reliable, and efficient route for synthesis of silver nanoparticles. Twenty one different typesof bacteria have been isolated from the twelve industrial waste water effluents. Among twenty one types of bacteria, *Bacillus pumillis* showed the highest resistance to silver. Silver nanoparticles were synthesized using different methods and silver precursor. In this research, we have found the first time the use of *Bacillus pumillis* for synthesis of silver nanoparticles. The synthesized silver nanoparticles were of rod and oval shaped and the estimated sizes were 5-93 nm. The size were within the nanometer range though the nanoparticles were surrounded by a thin layer of proteins and metabolites etc.., which were found from characterization using UV-Vis spectrophotometer, TEM, DLS,PSA, FTIR techniques. In all these techniques, it was proved that the concentration of bacteria, bacterialsupernatant and metal ion precursor play an important role in size and shape of silver nanoparticles. As the silver nanoparticles were synthesized only in the presence of light, it can be concluded that bacterial enzymes present in supernatant require light to be activate. As the concentration of metal ion was increased, the nanoparticle size and absorbance was also increased. Due to high solubility in water and easy reduction, silver nitrate is proven to be efficient precursor than silver sulfate. From technological and experimental point of view these silver nanoparticle have potential application to enhance bioremediation for Congo red dye removal up to 13 % due to advanced properties. While silver nanoparticles showed lowest dye removal capacity. The results revealed from the present investigation indicate that this nano bioremediation procedure has several advantages such as simple, cost-effectiveness, compatibly as well as applied to large scale.

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